Focused Ultrasound BBB Opening Yields Distinct, Tissue-Specific Extracellular Vesicle Profiles for Glioblastoma Liquid Biopsy

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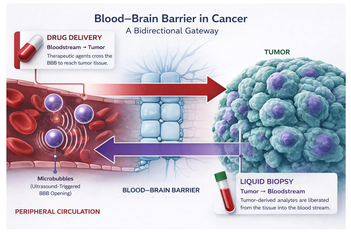
Introduction

- **Glioblastoma (GBM)** is the most common and lethal primary brain malignancy in adults with a 5-year survival rate of just 5-8%.
- The anatomically sequestered intracranial location of GBM and the **blood-brain barrier (BBB)** limit access to tumor-derived biomarkers in the circulation.
- **Extracellular vesicles (EVs)** are information-dense liquid biopsy analytes that carry proteins, RNAs, lipids, and metabolites reflective of parent cells.
- In neuro-oncology, EVs are emerging as promising **non-invasive biomarkers** for GBM characterization.
- **Focused ultrasound (FUS)** enables spatially precise, non-invasive, non-ionizing, and transient **BBB opening** when paired with microbubbles (MBs).
- **FUS-mediated BBB opening** is being actively explored in clinical trials for GBM therapy and liquid biopsy.
- Prior work suggests FUS can **increase GBM-derived EV release** and **alter EV cargo**, but these effects remain incompletely defined.

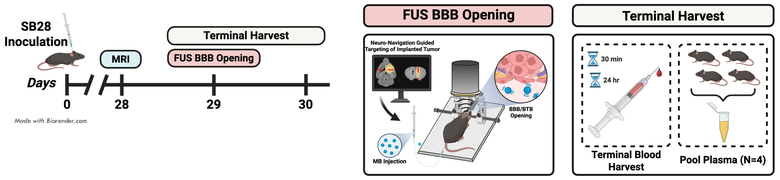


Transcranial focused ultrasound (FUS) is a disruptive, emerging solution to the problem of poor drug penetrance and low liquid biopsy yield within CNS tumors. MBs oscillate within a localized acoustic field, imparting mechanical stresses on the vasculature that yield transient "opening" of the BBB, predominantly via tight junction disruption.

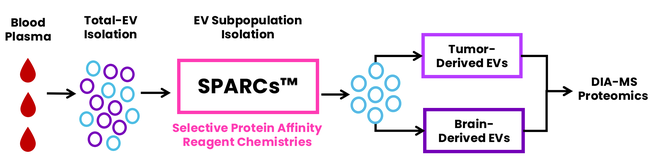
Knowledge gap: It remains unknown how FUS-mediated BBB opening shapes compartment-specific EV release kinetics and cargo profiles in brain tumors. Defining these effects is essential for identifying GBM-relevant circulating EV biomarkers uniquely enriched or differentially expressed after FUS.

Study objective: To investigate the impact of FUS-mediated BBB opening on the profiles of EVs associated with GBM and surrounding brain parenchyma, enriched using SPARCs™ technology.

Methods



FUS BBBO Procedure. Orthotopic SB28-bearing mice were treated with transcranial low-intensity, pulsed FUS in conjunction with i.v. MBs. FUS targeting was performed stereotactically via co-registration of pre-treatment MRIs with Allen Mouse Atlas. Acoustic emissions were monitored via real-time passive cavitation detection (PCD). All mice received controlled anesthesia exposure consisting of 0.7% isoflurane at 1 L/min during sham or FUS treatment. Mice were euthanized and plasma was collected either 30 mins or 24 hours post-FUS to investigate circulating EV populations within the window of either disrupted (30 mins) or resealed (24 hrs) BBB. Mouse plasmas across volume- and acoustic emissions-matched tumors were pooled (5 pools per group with n=4 mice per pool) to facilitate Tumor- and Neuro- SPARCs™ EV isolation.



SPARCs Enrichment. Total EVs were isolated via ion-exchange chromatography and characterized in concordance with the Minimal Information for Studies of Extracellular Vesicles (MISEV) 2023 guidelines. EVs were incubated with TumorSPARCs and NeuroSPARCs to enrich tumor-derived EVs and brain-derived EVs, respectively. SPARCs-enriched EV protein was subjected to DIA-MS on an Orbitrap Astral (Thermo Fisher Scientific, Waltham, MA) at Cedars-Sinai Precision Biomarker Laboratories (Beverly Hills, CA).

Results

FUS BBB Opening Does Not Modulate Circulating EV Concentration, Size, or Protein Concentration

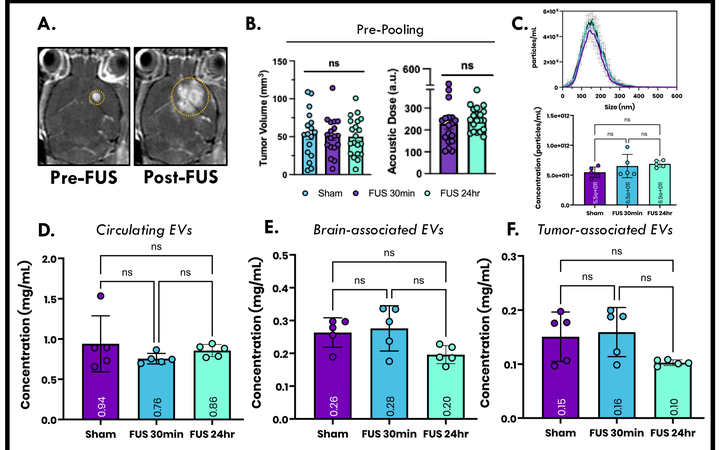


Figure 1. *Acute Assessment of Circulating EV Concentration, Size, and Protein Concentration following FUS BBB Opening.* **A.** Representative CE-T1w MRI of GBM (SB28) bearing mouse before and after FUS BBB opening. The dotted orange circle delineates the region of enhanced contrast penetration due to tumor and FUS-mediated BBB disruption. **B.** Tumor volumes and acoustic "dose" (resolved from PCD) for all tumors. **C.** Size distribution of total circulating EV populations (top) and total circulating EV concentrations, determined via nanoparticle tracking analysis (bottom). **D-F.** Protein concentrations within total circulating EVs (D), brain-associated EVs (E), and tumor-associated EVs (F) as measured via NanoOrange protein assay. Significance assessed via Kruskal-Wallis test. n.s. = not significant.

FUS BBB Opening Profoundly Alters EV Proteome and Elicits Unique Protein Content

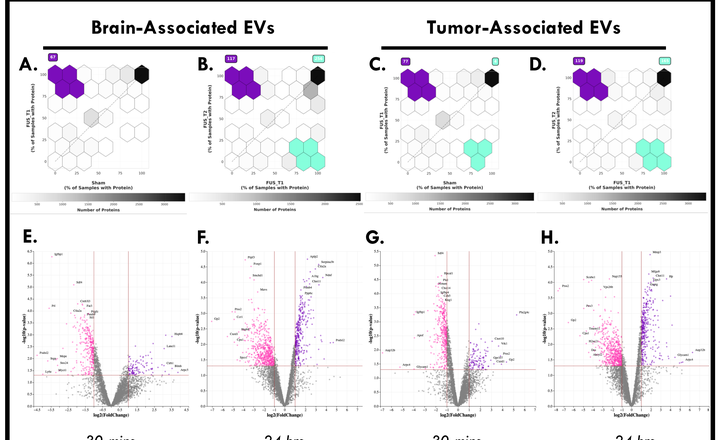


Figure 2. *Temporal Investigation of Circulating EV Proteome Following FUS BBB Opening.* **A-D.** Hexbin plots displaying proteins binned by the percentage of samples in which they were identified in each group. Bins are shaded by the number of protein overlap between groups. **E-H.** Volcano plots (FUS vs. Sham 30 mins, FUS 24 hrs vs 30 mins) displaying differentially expressed EV proteins enriched by NeuroSPARCs (E, F) and TumorSPARCs (G, H) across time points. Cutoff thresholds were defined as $p < 0.05$ and $\log_2$ fold-change +/- 1.

EV Proteome Reflects FUS-Induced Alterations in TME Stress, BBB Dynamics, and Neuro-Immune Cross-Talk

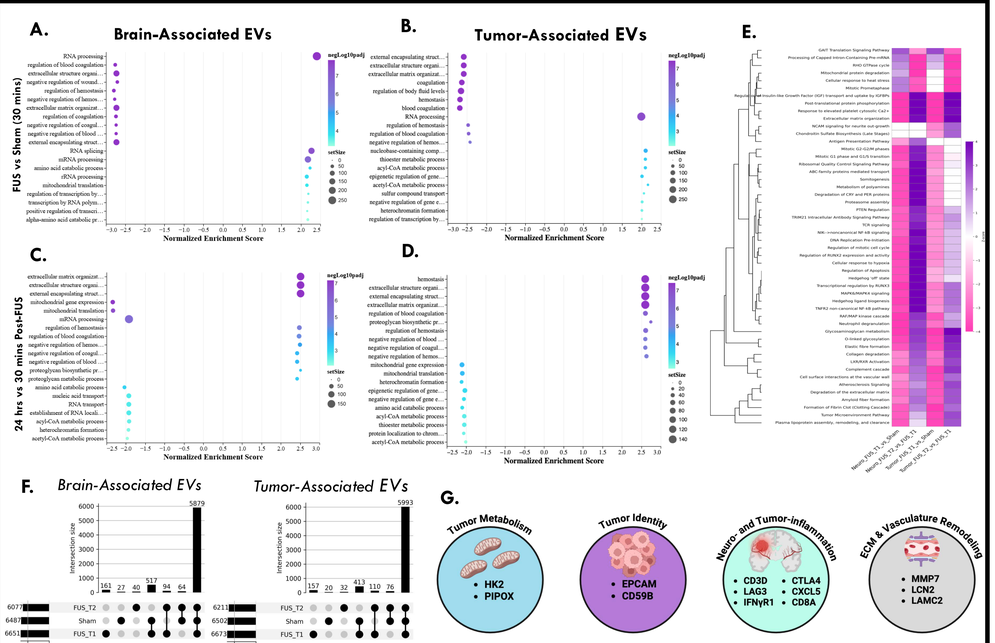
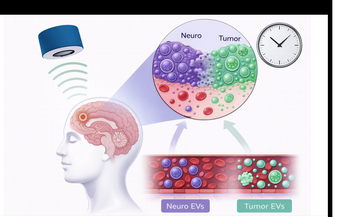


Figure 3. *Compartment-specific EV Pathway Analysis Following FUS BBB Opening.* **(A-D)** Pathway enrichment analysis of differentially abundant proteins within brain- or tumor-associated EVs. Pathway analysis was performed for FUS vs Sham conditions (30 mins; A, B) or longitudinally (24 hours vs 30 mins post-FUS; C, D). Top terms were sorted based on $-\log_{10}(p_{adj})$ in descending order. **(E)** Ingenuity Pathway Analysis for conditions depicted in A-D. Tile plot showing the top 50 significantly enriched pathways $|\log_2(\text{Fold-change})| > 1$, $p < 0.05$, z-score >= 2. **(F)** UpSet plot depicting protein counts with respect to experimental group for brain- (left) and tumor- (right) associated EVs. **(G)** Select unique protein cargo elicited by FUS within circulating brain- and/or tumor-associated EVs.

Conclusions

- FUS BBB opening drives robust, compartment-specific reprogramming of EV proteomes in GBM, revealing biomarker candidates absent under sham conditions.
- These findings position EV profiling as a sensitive approach for capturing FUS-induced tissue remodeling and may expand the biomarker repertoire available for spatially selective GBM liquid biopsy.
- Ongoing studies are assessing temporal dynamics and correspondence with parental tissue proteomes.



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